



PATENT
Docket No. 255.0004 0101

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Mark A. Sheridan et al.)	Group Art Unit:	1651
Serial No.:	09/727,739)	Examiner:	Unknown
Filed:	December 1, 2000)	Confirmation No.:	4181
For:	SOMATOSTATINS AND METHODS			

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington D.C. 20231

Sir:

Prior to taking up the above-identified patent application for examination, please enter the following amendments.

In The Specification

Please replace the paragraph at page 20, line 12 to page 21, line 9, with the following rewritten paragraph. Per 37 C.F.R §1.121, this paragraph is also shown in Appendix A, page A1, with notations to indicate the changes made.

A two-phase rapid amplification of cDNA ends (RACE) PCR-based approach (Fig. 4) was used for the isolation and characterization of selected cDNA sequences as described previously (Moore et al., *Gen. Comp. Endocrinol.*, 98, 253-261 (1995). In phase I, endogenous poly-A RNA was reverse transcribed from 15 µg of trout pancreatic total RNA with Superscript II reverse transcriptase (Gibco/BRL, Gaithersburg, MD) and a 37 nucleotide antisense adapter primer 5'-GGCCACGCGTCGACTAGTAC(T)₁₇-3' (SEQ ID NO:22) (Gibco/BRL). Five microliters of the reverse transcription reaction were used as template for 3'-RACE PCR with a 21-base somatostatin gene-specific primer 5'-AAGAACTTCTTCTGGAAGAC-3' (GSP-1; SEQ ID NO:25) and the universal amplification primer 5'-CUACUACUACUAGGCCACGCGTCGACTAGTAC-3'(UAP; SEQ ID NO:23). After an initial denaturation cycle of 94°C for 5 minutes, 35 PCR

cycles were performed, each consisting of 1-minute annealing (42°C), 1-minute extension (72°C), and 1-minute denaturation (94°C). In the last cycle, the extension time was increased to 10 minutes to ensure complete extension. The resulting PCR product (350 bp) was identified by electrophoresis on an agarose gel containing 1% (w/v) agarose (Gibco/BRL) and 1% (w/v) NuSeive GTG agarose (FMC Bioproducts, Rockland, ME) in 1X TBE Buffer, followed by ethidium bromide staining and UV transillumination. Amplified fragments were directly cloned into the TA cloning vector *PCR 2000* (Invitrogen, San Diego, CA). Positive colonies were identified by agarose gel electrophoresis of restriction enzyme digests (*Eco*RI; Promega, Madison, WI) of purified plasmid preparations (Del Sal et al., BioTech., 7, 514-519 (1989)). One to 2 µg of plasmid were denatured and sequenced by the dideoxy chain-termination method (Sequenase Kit; U.S. Biochemicals Corp., Cleveland, OH) according to the manufacturer's protocol. All sequences were confirmed by sequencing multiple colonies from at least three independent PCR reactions and with two or more different primers in both directions, with dGTP dideoxy nucleotides. Sequencing gels were made with 30% formamide to eliminate the possibility of G/C compressions.

Please replace the paragraph at page 39, line 16 to line 19, with the following rewritten paragraph. Per 37 C.F.R §1.121, this paragraph is also shown in Appendix A, page A3, with notations to indicate the changes made.

The results, shown in Fig. 8, indicate that the human somatostatin receptor type 1 has a greater affinity for salmonid SS-25 (SEQ ID NO:16) than for either mammalian SS-14 (SEQ ID NO:1) or mammalian SS-28 (SEQ ID NO:21).

Remarks

These amendments simply correct typographical errors and add no new matter to the specification.

The amendments made to page 20, introduce a subscript into one nucleotide sequence at line 18, and eliminate an inadvertent space in another nucleotide sequence at line 22.

The amendment to page 39, line 19 is made to correctly identify the sequence identification number. This sequence identification number is identified at page 16, line 29 of the specification.

The Examiner is invited to contact Applicants' Representatives, at the below-listed telephone number, if it is believed that prosecution of this application may be assisted thereby.

CERTIFICATE UNDER 37 C.F.R. 1.10:

"Express Mail" number: EL 776900900 US
Date of Deposit: April 24, 2001

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR §1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, **ATTN: Box Missing Parts**, Washington, D.C. 20231.

By: Kimberly A. Hayes

Name: Kimberly A. Hayes

April 24, 2001

Date

VAS:lmg

Respectfully submitted,

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Appendix A

Specification Amendments with Notations to Indicate Changes Made

Applicants: Mark A. Sheridan et al.

Serial No.: 09/727,739

Filed: December 1, 2000

For: SOMATOSTATINS AND METHODS

Deletions are bracketed, additions are underlined, and all changes are highlighted in gray.

Page 20, line 12 to page 21, line 9

A two-phase rapid amplification of cDNA ends (RACE) PCR-based approach (Fig. 4) was used for the isolation and characterization of selected cDNA sequences as described previously (Moore et al., Gen. Comp. Endocrinol., 98, 253-261 (1995)). In phase I, endogenous poly-A RNA was reverse transcribed from 15 µg of trout pancreatic total RNA with Superscript II reverse transcriptase (Gibco/BRL, Gaithersburg, MD) and a 37 nucleotide antisense adapter primer 5'-GGCCACGCGTCGACTAGTAC(T)[17]12-3' (SEQ ID NO:22) (Gibco/BRL). Five microliters of the reverse transcription reaction were used as template for 3'-RACE PCR with a 21-base somatostatin gene-specific primer 5'-AAGAACTTCTTCTGGAAGAC-3' (GSP-1; SEQ ID NO:25) and the universal amplification primer

5'-CUACUACUACUAGGCCACGCGTCGACTAGTAC-3'

5'-CUACUACUACUAGGCCACGCGTCGACTAGTAC-3'(UAP; SEQ ID NO:23). After an initial denaturation cycle of 94°C for 5 minutes, 35 PCR cycles were performed, each consisting of 1-minute annealing (42°C), 1-minute extension (72°C), and 1-minute denaturation (94°C). In the last cycle, the extension time was increased to 10 minutes to ensure complete extension. The resulting PCR product (350 bp) was identified by electrophoresis on an agarose gel containing 1% (w/v) agarose (Gibco/BRL) and 1% (w/v) NuSeive GTG agarose (FMC Bioproducts, Rockland, ME) in 1X TBE Buffer, followed by ethidium bromide staining and UV transillumination. Amplified fragments were directly cloned into the TA cloning vector *PCR 2000* (Invitrogen, San Diego, CA). Positive colonies were identified by agarose gel electrophoresis of restriction enzyme digests (*EcoRI*; Promega, Madison, WI) of purified plasmid preparations (Del Sal et al., BioTech., 7, 514-519 (1989)). One to 2 µg of plasmid were

Preliminary Amendment - Appendix A

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Specification Amendments with Notations to Indicate Changes Made

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Title: *SOMATOSTATINS AND METHODS*

denatured and sequenced by the dideoxy chain-termination method (Sequenase Kit; U.S. Biochemicals Corp., Cleveland, OH) according to the manufacturer's protocol. All sequences were confirmed by sequencing multiple colonies from at least three independent PCR reactions and with two or more different primers in both directions, with dGTP dideoxy nucleotides. Sequencing gels were made with 30% formamide to eliminate the possibility of G/C compressions.

Preliminary Amendment - Appendix A

Page A3

Specification Amendments with Notations to Indicate Changes Made

Serial No. 09/727,739

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Title: SOMATOSTATINS AND METHODS

Page 39, line 16 to line 19

The results, shown in Fig. 8, indicate that the human somatostatin receptor type 1 has a greater affinity for salmonid SS-25 (SEQ ID NO:16) than for either mammalian SS-14 (SEQ ID NO:1) or mammalian SS-28 (SEQ ID NO:[53]21).